



Seminal Fluid Analysis in Iraqi Fertile and Infertile Males Defined by the World Health Organization Criteria of 2010

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Abstract

A total of 116 males with primary infertility and 32 fertile males (normozoospermia; NOR) were studied to evaluate parameters of seminal fluid analysis. Based on WHO criteria of 2010 for general seminal fluid analysis, the patients were distributed into three groups: 32 azoospermic (AZO), 40 oligozoospermic (OLI) and 44 asthenozoospermic (AST) patients. AZO and OLI patients and NOR shared an approximated mean of seminal fluid volume (2.25, 2.75 and 2.50 ml, respectively), while it was significantly increased (3.58 ml) in AST patients. In NOR men, the spermatozoa concentration was 65.13×10^6 spermatozoa/ml, while it was significantly decreased in AST (51.42×10^6 spermatozoa/ml) and OLI (5.58×10^6 spermatozoa/ml) patients. The percentage frequency of progressive motility was significantly decreased in OLI and AST patients as compared to NOR (9.6 and 16.3, respectively vs. 50.6%). In contrast, the non-progressive motile (45.4 and 30.4, respectively vs. 21.9%) or immotile (45.0 and 52.5, respectively vs. 27.5%) spermatozoa were significantly increased in the patients. OLI and AST patients shared an approximated mean of abnormal spermatozoa frequency (56.7 and 58.3%, respectively), but both frequencies were significantly higher than the observed frequency in NOR (23.1%).

Key words: male infertility, normozoospermia, azoospermia, oligozoospermia, asthenozoospermia.

Introduction

Semen analysis is the method of choice in assessing male infertility. During ejaculation, semen is produced from a concentrated suspension of spermatozoa, stored in the paired epididymides, mixed with, and diluted by, fluid secretions from the accessory sex organs. Comparison of pre- and post-vasectomy semen volumes reveals that about 90% of semen volume is made up of secretions from the accessory organs, mainly the prostate and seminal vesicles, with minor contributions from the bulbourethral (Cowper's) glands and

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epididymides [1]. Semen has two major quantifiable attributes: the first is total number of spermatozoa and this reflects sperm production by the testes and the patency of the post-testicular duct system, while the second is total fluid volume contributed by various accessory glands and this reflects the secretory activity of the glands. The nature of spermatozoa (their vitality, motility and morphology) and the composition of seminal fluid are also important for sperm function [2]. Under given conditions of collection, semen quality depends on factors that usually cannot be modified, such as sperm production by the testes, accessory organ secretions and recent (particularly febrile) illness, as well as other factors, such as abstention time, that should be recorded and taken into account in interpreting the results [3].

The 'WHO Manual for the Examination of Human Semen and Sperm (semen)-cervical mucus interaction' [4-6] is widely used as a source of standard methodology for laboratories engaged in semen analyses. However, the interpretation and application of previous WHO 'normal' or 'reference' values for semen parameters used thus far have limitations, since the data were derived from imprecisely defined reference populations and obtained from laboratories with unknown comparability with respect to analytical methodologies. These values were limited by the lack of available data on semen variables in recent fathers, and did not define true reference ranges or limits. There have been no consensus around the suitability of these values, as some centers consider the cited values for characteristics of sperm concentration, morphology and motility too high, whereas others consider them too low [7]. The latter group of investigators (Cooper *et al.*, 2010) obtained semen samples from over 4500 men in 14 countries on four continents from retrospective and prospective analyses on fertile men, men of unknown fertility status and men selected as normozoospermic. Accordingly, they generated that the lower reference limit for semen variables is semen volume, 1.5 ml (1.4-1.7 ml); total sperm number, 39 million per ejaculate (33-46 million); sperm concentration, 15 million per ml (12-16 million); vitality, 58% live (55-63%); progressive motility, 32% (31-34%); morphologically normal forms, 4.0% (3.0-4.0%). Based on these finding, the WHO has changed the reference ranges used in semen analyses and accordingly it issued a new definition of semen variables [8]. Accordingly, the present investigation initiated the scope to define fertile and infertile groups of Iraqi population.

Subjects, Materials and Methods

Subjects: A total of 116 males with primary infertility attending Kamal Al-Samaraie Hospital, Centre of Infertility and *in vitro* Fertilization (Baghdad) and Baghdad Teaching Hospital (Infertility Clinic) during the period March-August 2010 were enrolled in this study. They were clinically examined and evaluated by the consultant medical staff at the two hospitals. The patients were Iraqi Arabs and their age mean \pm S.E. was 30.3 ± 0.7 years. In addition, 32 fertile males (normozoospermia; NOR), matched patients for ethnicity and age (31.4 ± 1.1 years), were also included in the study, and they were husbands of wives who had fertility complications. Based on clinical examination and seminal fluid analysis and according to the recommendations of World Health Organization [8], patients were distributed into three clinical groups: azoospermia (AZO; 32 patients and their age was 28.2 ± 0.7 years), oligozoospermia (OLI: 40 patients and their age was 31.3 ± 1.3 years) and asthenozoospermia (AST: 44 patients and their age was 33.4 ± 1.0 years).

Seminal Fluid Collection and Examination: Seminal fluid was obtained by masturbation after 3-5 days of sexual abstinence. The samples were collected in sterile, wide mouthed, non-toxic container and processed in the laboratory within an hour of ejaculation. In accordance with the WHO manual of seminal fluid examination [8], the seminal fluid was evaluated in terms of macroscopical (appearance, volume, liquefaction, pH and viscosity) and microscopical (spermatozoa concentration, morphology and motility) examinations. Motility of spermatozoa was assessed directly after liquefaction, by applying a drop of a gently mixed seminal fluid on a clean slide and covered with a cover slip. After two minutes, the slide was inspected microscopically to determine the type of motility, and at least eight fields were examined. Three categories of spermatozoa movement were adopted: progressive motility (PR: spermatozoa moving actively, either linearly or in a large circle, regardless of speed). Non-progressive motility (NP: all other patterns of motility with an absence of progression, e.g. swimming in small circles, the flagellar force hardly displacing the head, or when only a flagellar beat can be observed). Immotility (IM: no movement).

Statistical Analysis: Data were presented in terms of means \pm standard errors (S.E.), and differences between means were assessed by Duncan's tests. The difference was considered significant when the probability (P) value was ≤ 0.05 . In further analyses, Pearson's Chi-square was also adopted. The analysis was carried out using the computer programme SPSS (Statistical Package for Social Sciences) version [13].

Results and Discussion

Appearance of Seminal Fluid: In most cases, the normal seminal fluid had a homogenous grey-opalescent appearance, but it was less opaque when spermatozoa concentration was low as in OLI patients, or the spermatozoa were not detected as AZO patients.

Volume: The AZO and OLI patients, as well as, controls shared an approximated mean of seminal fluid volume (2.25, 2.75 and 2.50 ml, respectively), while it was significantly ($P \leq 0.05$) increased (3.58 ml) in AST patients as compared with AZO patients and NOR, but not with OLI patients (Table 1).

Although some significant differences were observed in the volume of seminal fluid between the investigated groups of infertility and NOR, the means are still higher than the WHO stricter guidelines for how to recognize an appropriate sperm volume that is stated at approximately 2.0 ml [8]. Considering such parameter alone may underestimate its significance, because it reflects testicular volume, and thus is a measure of total testicular sperm output, which is directly related to the chances of pregnancy after coitus [9]. Further more, the volume of the ejaculate is contributed mainly by the seminal vesicles and prostate gland, with a small amount from the bulbourethral glands and epididymides; therefore, precise measurement of volume is essential in any evaluation of semen, because it allows the total number of spermatozoa and non-sperm cells in the ejaculate to be calculated [3].

Liquefaction: Immediately after measuring the volume, the tube containing seminal fluid was incubated at 37°C, and kept under inspection for liquefaction every 15 minutes and up to 60 minutes. Initially, the semen was typically a semi-solid coagulated mass. Within the first 15 minutes, the semen usually began to liquefy (become thinner), and as liquefaction continued, the semen became more homogeneous and quite watery, and in the final stages only small

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areas of coagulation remain. In most enrolled cases, a complete liquefaction occurred within 15-30 minutes.

pH: The pH was measured after liquefaction at a uniform time (30 minutes) using a pH test paper, because it is influenced by the loss of CO₂ that occurs after production. In most cases, the pH was alkaline with the range 7.5-8.0.

Viscosity: After liquefaction, the viscosity of the sample was estimated by gently aspirating it into a wide-bore (approximately 1.5 millimeter diameter) plastic disposable pipette, allowing the semen to drop by gravity and observing the length of any thread. A normal sample leaves the pipette in small discrete drops, but if viscosity is abnormal, the drop will form a thread more than two centimeters long. The investigated cases had a normal viscosity.

Spermatozoa Concentration: In NOR, the spermatozoa concentration was 65.13×10^6 spermatozoa/ml, while it was significantly ($P \leq 0.05$) decreased in AST patients (51.42×10^6 spermatozoa/ml), as well as, OLI patients (5.58×10^6 spermatozoa/ml). The AZO patients showed no spermatozoa in their seminal fluid (Table 2).

The spermatozoa concentration is related to both time to pregnancy [10] and pregnancy rates [11] and is a predictor of conception [12]. The present definition of infertility groups (AZO, OLI and AST) is in agreement with the WHO guide that stated the lower reference limit in each group, and in addition, the NOR are also fit the obligation of such guide [8]. Therefore, the observed significant differences in spermatozoa concentration between OLI, AST and NOR are expected.

Spermatozoa Morphology: The OLI and AST patients shared an approximated mean of abnormal spermatozoa frequency (56.7% and 58.3%, respectively), but both frequencies were significantly ($P \leq 0.05$) higher than the observed frequency in NOR (23.1%), as shown in table 3A. However, when the absolute count of these abnormalities was considered a further observation was made, in which the highest count of spermatozoa abnormalities was observed in AST patients (30.1×10^6 spermatozoa/ml), followed by NOR (15.0×10^6 spermatozoa/ml) and finally OLI patients (3.0×10^6 spermatozoa/ml). These three means showed significant ($P \leq 0.05$) difference between them (Table 3B).

In term of percentage frequency, OLI and AST patients shared a similar an approximated frequency of morphologically abnormal spermatozoa, but the absolute count reshaped such observation, and the highest count was observed in AST patients, while the lowest count was recorded in OLI patients. Such difference is related to the spermatozoa concentration, because OLI demonstrated the lowest level of spermatozoa concentration (Table 2); therefore the percentage frequency evaluation is more informative in this regard; and abnormal spermatozoa morphology is suggested to impact fertility in both groups of infertility (OLI and AST). Accordingly, spermatozoa morphology evaluation is considered to be a highly subjective procedure and based on WHO criteria, a normal ejaculate must have at least 30% normal spermatozoa, and for the stricter criteria, fertile men must have greater than 14% normal forms in their semen and men with less than 4% of normal forms are considered subfertile [7,8]. However, Menkveld *et al.* [13] have recently criticized that measurement or evaluation and clinical significance of human spermatozoa morphology has always been and still is a controversial aspect of the semen analysis for the determination of a male's fertility potential. Such criticism is reasoned by the fact of the strict criteria definition and use of the criteria for spermatozoa morphology evaluation, but again they highlighted that sperm morphology measurement still has a very important role to play in the clinical evaluation of male fertility potential.

Spermatozoa Motility: The percentage frequency of PR motility was significantly ($P \leq 0.05$) decreased in OLI and AST patients as compared to NOR men (9.6 and 16.3, respectively vs. 50.6%). In contrast, the NP motile (45.4 and 30.4, respectively vs. 21.9%) or IM (45.0 and

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52.5, respectively vs. 27.5%) spermatozoa, were significantly increased in the patients (Table 4A). When the three types of spermatozoa motility was considered in terms of an absolute count, a more pronounced findings were gathered. With respect to PR motility, the spermatozoa absolute count showed a gradual decrease in NOR and AST and OLI patients ($33.0, 8.7$ and 0.8×10^6 spermatozoa/ml, respectively), and the difference between these means was significant ($P \leq 0.05$). In NP motility, the NOR and AST patients shared an approximated absolute mean count (14.3 and 15.9×10^6 spermatozoa/ml, respectively), but both of them were significantly ($P \leq 0.05$) higher than the observed mean in OLI patients (2.8×10^6 spermatozoa/ml). When immotility was inspected, AST patients showed the highest mean count (26.8×10^6 spermatozoa/ml), followed by NOR (17.9×10^6 spermatozoa/ml) and finally OLI patients (2.1×10^6 spermatozoa/ml), and the difference between these means was significant ($P \leq 0.05$), as shown in table 4B.

The present findings of PR motility (in term of percentage frequency or an absolute count) in OLI and AST patients strongly suggest that spermatozoa motility is an important factor that is related to male fertility. Furthermore, it has been suggested that spermatozoa motility assessment can be used more directly to address problems affecting the man and his reproductive organs. The presence of inflammatory cells (often peroxidase-positive granulocytes) suggests an ongoing inflammatory reaction and in addition, spermatozoa exposed to seminal vesicular fluid show decreased motility, survival and protection of the sperm chromatin [14], indicating that an abnormal sequence of ejaculation can cause decreased sperm function. Reduced spermatozoa motility can therefore be a symptom of disorders related to male accessory sex gland secretion and to the sequential emptying of these glands [15].

In conclusion, such study provides additional information to characterize relationships between semen parameters and male fertility. These data provide information on the value of semen quality measures to predict fertility in populations, and can be used to estimate decrements in fertility for risk assessment, but further investigations are certainly required and larger samples can further define the relationship between seminal fluid parameters and male infertility in Iraqi populations.

References

1. Duncan, W.W. and Thompson, H.S. (2007) Proteomics of semen and its constituents. *Proteomics Clinical Application*, 1: 861-875.
2. Björndahl, L. and Kvist, U. (2003) Sequences of ejaculation affects the spermatozoon as a carrier and its message. *Reproductive Biomedicine Online*, 7: 440-448.
3. Cooper, T.G.; Barfield, J.P. and Yeung, C.H. (2005) Changes in osmolality during liquefaction of human semen. *International Journal of Andrology*, 28: 58-60.
4. Comhaire F.; de Kretser, D.M. and Farley, T.M. WHO (1987). Towards more objectivity in diagnosis and management of male infertility. *International Journal of Andrology*, Suppl.7: 22-24.
5. The influence of varicocele on parameters of fertility in large group of men presenting to infertility clinics. World Health Organization, *Fertility and sterility*, WHO (1992). 57: 1289-1293.
6. Laboratory manual for the examination of human semen and sperm-cervical mucus interaction, fourth edition. Cambridge, Cambridge University Press. WHO (1999).
7. Cooper, T.G.; Noonan E., von Eckardstein S., Auger J., Baker H.W.G., Behre H.M., Haugen T.;B., Kruger T.; Wang, C., Mbizvo, M.T. and Vogelsong, K.M. (2010) World health organization reference values for human semen characteristics. *Human Reproduction Update*, 16: 231-245.

8. Laboratory Manual for the Examination and Processing of Human Semen, 5th edition. WHO (2010).
9. Cooper, T.G.; Noonan, E.; von Eckardstein, S.; Auger, J.; Baker H.W.G.; Behre H.M.; Haugen T.;B., Kruger T., Wang C., Mbizvo M.T. and Vogelsong K.M.(2010). World health organization reference values for human semen characteristics. *Human Reproduction Update*, **16**: 231-245.
10. Slama ,R.;Eustache, F.; Ducot, B.; Jensen, T.K.; Jørgensen, N.; Horte ,A.; Irvine, S.; Suominen, J.; Andersen, A.G.; Auger, J.; Vierula ,M.; Toppari ,J.; Andersen ,A.N.; Keiding N., Skakkebaek N.E., Spira A. and Jouannet P. (2002) Time to pregnancy and semen parameters: a cross-sectional study among fertile couples from four European cities. *Human Reproduction*, **17**: 503-515.
11. Zinaman, M.J.; Brown ,C.C.; Selevan, S.G. and Clegg ,E.D. (2000) Semen quality and human fertility: a prospective study with healthy couples. *Journal of Andrology*, **21**: 145-153.
12. Larsen, L.; Scheike ,T.; Jensen, T.K.; Bonde, J.P.; Ernst, E.; Hiollund, N.H.; Zhou, Y.; Skakkebaek N.E. and Giwercman A. (2000) Computer-assisted semen analysis parameters as predictors for fertility of men from the general population. The Danish First pregnancy planner study team. *Human Reproduction*, **15**: 1562-1567.
13. Menkveld, R.; Holleboom ,C.A.G. and Rhemrev, J.P.T. (2011) Measurement and significance of sperm morphology. *Asian Journal of Andrology*, **13**: 59-68.
14. Jequier, A.M. (2006) The importance of diagnosis in the clinical management of infertility in the male. *Reproductive Biomedicine Online*, **13**: 331-335.
15. Bjorndahl, L. (2010) The usefulness and significance of assessing rapidly progressive spermatozoa. *Asian Journal of Andrology*, **12**: 33-35.

Table(1): Volume of seminal fluid in infertile patients (azoospermia, oligozoospermia and asthenozoospermia) and normozoospermia.

Groups	No.	Mean ± S.E. (ml)
Azoospermia	32	2.25 ± 0.13 ^B
Oligozoospermia	40	2.75 ± 0.26 ^{AB}
Asthenozoospermia	44	3.58 ± 0.48 ^A
Normozoospermia	32	2.50 ± 0.18 ^B

Different letters: Significant difference ($P \leq 0.05$) between means.

Table(2): Spermatozoa concentration in infertile patients (azoospermia, oligozoospermia and asthenozoospermia) and normozoospermia.

Groups	No.	Mean ± S.E. (Spermatozoa x 10 ⁶ /ml)
Azoospermia	32	0.00 ± 0.00
Oligozoospermia	40	5.58 ± 0.62 ^C
Asthenozoospermia	44	51.42 ± 1.94 ^B
Normozoospermia	32	65.13 ± 0.88 ^A

Different letters: Significant difference ($P \leq 0.05$) between means.

Table(3A): Spermatozoa abnormality (%) in infertile patients (oligozoospermia and asthenozoospermia) and normozoospermia.

Groups	No.	Mean \pm S.E. (%)
Oligozoospermia	40	56.7 \pm 3.0 ^A
Asthenozoospermia	44	58.3 \pm 3.0 ^A
Normozoospermia	32	23.1 \pm 0.6 ^B

Different letters: Significant difference ($P \leq 0.05$) between means.

Table(3B): Spermatozoa abnormality (absolute count) in infertile patients (oligozoospermia and asthenozoospermia) and normozoospermia.

Groups	No.	Mean \pm S.E. (Spermatozoa $\times 10^6$ /ml)
Oligozoospermia	40	3.0 \pm 0.3 ^C
Asthenozoospermia	44	30.1 \pm 2.1 ^A
Normozoospermia	32	15.0 \pm 0.4 ^B

Different letters: Significant difference ($P \leq 0.05$) between means.

Table(4A): Spermatozoa motility (percentage frequency) in infertile patients (oligozoospermia and asthenozoospermia) and normozoospermia.

Groups	No.	Motility (Mean \pm S.E.; %)		
		Progressive	Non-progressive	Immotile
Oligozoospermia	40	9.6 \pm 2.6 ^C	45.4 \pm 4.6 ^A	45.0 \pm 4.5 ^A
Asthenozoospermia	44	16.3 \pm 2.2 ^B	30.4 \pm 2.7 ^B	52.5 \pm 3.6 ^A
Normozoospermia	32	50.6 \pm 0.4 ^A	21.9 \pm 0.9 ^C	27.5 \pm 0.9 ^B

Different letters: Significant difference ($P \leq 0.05$) between means of columns.

Table(4B): Spermatozoa motility (absolute count) in infertile patients (oligozoospermia and asthenozoospermia) and normozoospermia.

Groups	No.	Motility (Mean \pm S.E.; spermatozoa/ml $\times 10^6$)		
		Progressive	Non-progressive	Immotile
Oligozoospermia	40	0.8 \pm 0.2 ^C	2.8 \pm 0.4 ^B	2.1 \pm 0.2 ^C
Asthenozoospermia	44	8.7 \pm 1.4 ^B	15.9 \pm 1.6 ^A	26.8 \pm 2.1 ^A
Normozoospermia	32	33.0 \pm 2.6 ^A	14.3 \pm 0.7 ^A	17.9 \pm 0.5 ^B

Different letters: Significant difference ($P \leq 0.05$) between means of columns.

تحليل السائل المنوي في ذكور عراقيين خصبين و عقيمين وفقاً لمعايير منظمة الصحة العالمية لعام 2010

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الخلاصة

درس 116 من الذكور المصابين بالعقم الأولي و32 من الذكور الخصبة لتقييم معايير تحليل السائل المنوي. وفي ضوء معايير منظمة الصحة العالمية لعام 2010 لتحليل السائل المنوي، وزع المرضى على ثلاث مجاميع وهي اللانطفية (32 مريضاً) وقلّة النطف (40 مريضاً) ووهن النطف (44 مريضاً). تقارب معدل حجم السائل المنوي في مرضى اللانطفية وقلّة النطف وكذلك في الذكور الخصبة (2.25 و2.75 و2.50 مل، على التوالي)، ولكنه ارتفع معنوياً في مرضى وهن النطف (3.58 مل). كان تركيز الحيوانات المنوية في الذكور الخصبة 65.13 مليون حيوان منوي/مل، بينما انخفض هذا التركيز معنوياً في مرضى وهن النطف (51.42 مليون حيوان منوي/مل) وقلّة النطف (5.58 مليون حيوان منوي/مل). انخفضت معنوياً النسبة المئوية للحركة التقدمية في مرضى قلّة النطف و وهن النطف مقارنة بالذكور الخصبة (9.6 و16.3، على التوالي مقابل 50.6%)، وعلى العكس من ذلك ارتفعت معنوياً الحيوانات المنوية اللاتقدمية (45.4 و30.4، على التوالي مقابل 21.9%) أو غير المتحركة في المرضى (45.0 و52.5، على التوالي مقابل 27.5%). تقارب معدل تكرار الحيوانات المنوية غير الطبيعية في مرضى قلّة النطف ووهن النطف (56.7 و58.3%)، على التوالي، إلا أن كلا التكرارين ارتفعا معنوياً مقارنة بالتكرار المشاهد في الذكور الخصبة (23.1%).

الكلمات المفتاحية : male infertility, normozoospermia, azoospermia, oligozoospermia, asthenozoospermia .